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Dated: September 27, 2006 Signature: Valerie Cohen
(Valerie Cohen)

PATENT
Docket No. 514162000120

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Che-Kun James SHEN

Serial No.: 10/014,220

Filing Date: November 9, 2001

For: HS-40 ENHANCER-CONTAINING VECTORS IN TRANSGENIC ANIMALS

Examiner: S. Kaushal

Group Art Unit: 1633

DECLARATION OF CHE-KUN JAMES SHEN PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Che-Kun James Shen, declare as follows:

1. I am currently employed as a Distinguished Research Fellow and Director at the Institute of Molecular Biology, Academia Sinica.

2. I am the inventor of the invention disclosed in the above-referenced patent application, and am familiar with the contents thereof. I have assigned my rights in the invention to the Academia Sinica and stand to receive 20% of profits in connection with the invention pursuant to my employment with Academia Sinica.

3. I received a Ph.D. in Chemistry from the University of California, Berkeley, July, 1977, and have been actively involved in molecular biology and biotechnology-related research for 30 years.

4. I am a co-author of Zhang *et al.* (JBC 270(15):8501-8505, 1995) and therefore I am familiar with the contents thereof. In addition, I have read the Office Action dated October 19, 2005 where the Examiner discussed this article. The transfection assays that we conducted were transient transfection assays. In a transient transfection assay, the DNA construct does not integrate into the host cell's genome. **This is particularly true in mammalian cells such as human cells because the random integration frequency in mammalian cells is very low under the conditions used for the transfection assay, in the range of one event per 10^2-10^4 cells (Roth, D.B. and Wilson, J. H. p.621-651, Genetic Recombination, Am. Soc. Microbiol. 1988). Further the time after transient transfection until assaying was too short (five days) to allow the random integration to occur. Thus, no cells would have been stably transfected within the time period of the assay and even if the cells were maintained for a longer period of time, only a very small fraction of the cells would have been stably transfected.** Even when conditions are optimized to promote integration, the efficiency is still quite low. To overcome this low efficiency, scientists attempting to achieve integration of a vector use a selectable marker to kill cells that do not have the vector integrated into their genome. We did not perform any such selection step for the paper. In addition, the vector used for transient transfection did not have a selectable marker on it that could have been used to select for integration. Finally, the vector used was circular when electroporated. Circular vectors integrate with lower efficiency that linearized vectors.

5. The TCTGAGTCA sequence provides the unexpected characteristic of position independent expression when integrated into the genome. Position independence can only be demonstrated when an expression construct is integrated into the genome of the host cell, not during transient expression assays. Therefore, position independent expression was not seen in our experiments for the Zhang *et al.* paper and would not have been predicted from the results that we published in Zhang *et al.* Furthermore, as demonstrated in our patent application, the wild-type sequence does not provide position independent expression. If the wild-type enhancer sequence did not provide position independent expression, then one of skill in the art would assume that any mutant sequence would not provide position independent expression. It is a completely unexpected result for the mutant sequence TCTGAGTCA to have gained this novel characteristic of position independent expression. One skilled in the art would have not

predicted that this sequence provides position independent expression until reading our patent application and the results therein.

September 27, 2006

Che-Kun James Shen